DRG® MPO (Myeloperoxidase) ELISA (EIA-4571)

REVISED 18 MAY 2006 **RUO**

1 INTENDED USE

DRG® MPO (Myeloperoxidase) ELISA provides a method for the quantitative determination of human Myeloperoxidase (MPO) in serum and plasma.

2 SUMMARY AND EXPLANATION OF THE TEST

Myeloperoxidase (MPO), an iron containing glycoprotein, is a covalently bounded tetrameric complex with a molecular weight of 150 kDa. It is composed of two glycosylated alfa chains of MW 59-64 kDa and two unglycosylated beta chains of MW 14 kDa. MPO is found in abundance in the primary azurophilic granules of neutrophils and is present in monocytes.

In response to microbial invasion, MPO is released from the cytoplasmic granules of neutrophilis into the phagasome and extracellular space, catalyzing the conversion of hydrogen peroxide and chloride ions (Cl) into hypochlorous acid, a potent oxidant agent.

Myeloperoxidase traditionaly is used as a marker of airway inflammation caused by asthma or environmental irritants. It is also believed that MPO participates in different stages of atherogenesis and has a potential role in the promotion of atherosclerosis. Association between elevated MPO levels in serum and cardiovascular disease (CAD) supports an important role for MPO as an inflammatory marker in CAD, making it possible to identify patients at risk for cardiac events in the absence of mycardial necrosis.

3 PRINCIPLE OF THE PROCEDURE

DRG® MPO ELISA is a solid phase two-site enzyme immunoassay. It is based on the sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the MPO-molecule.

During incubation, MPO in the sample react with anti- MPO antibodies bound to microtitration well. After washing, peroxidase conjugated anti-MPO antibodies are added and after the second incubation and a simple washing step that removes unbounded enzyme labelled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

4 WARNINGS AND PRECAUTIONS

- **For Research Use only. Not for Use in Diagnostic Procedures.** Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H_2SO_4. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

5 MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200, 250 and 500 µl (repeat pipettes preferred for addition of Enzyme Conjugate Solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation

- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

6 REAGENTS

Each MPO ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one calibration curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical Lot numbers.

The expiry date for the complete kit is stated on the outer label.

The recommended storage temperature is +2-8°C.

Coated Plate (Mouse monoclonal anti-MPO)	1 plate	96 wells (8-well strips)	Ready for use
For unused microtitration wells, completely reseal the bag using adhesive tape and use within two months.			
Standard Concentration indicated on vial label.	5 vials	1 ml	Ready for use
Sample Buffer Colour coded yellow	1 vial	12 ml	Ready for use
Assay Buffer Colour coded red	1 vial	12 ml	Ready for use
Enzyme Conjugate 11X (Peroxidase conjugated mouse monoclonal anti-MPO). *Note! Light sensitive!*	1 vial	1.2 ml	Preparation, see below
Enzyme Conjugate Buffer Colour coded blue	1 vial	12 ml	Ready for use
Wash Buffer 21X Storage after dilution: +2-8°C for 4 weeks.	1 bottle	40 ml	Dilute with 800 ml redistilled water to make Wash Buffer.
Substrate TMB (TMB) Colourless solution. *Note! Light sensitive!*	1 vial	22 ml	Ready for use
Stop Solution 0.5 M H_2SO_4	1 vial	7 ml	Ready for use

6.1 Preparation of Enzyme Conjugate Solution

Prepare the needed volume of Enzyme Conjugate Solution by dilution of Enzyme Conjugate 11 X, (1+10) in Enzyme Conjugate Buffer or according to the table below. Mix gently.

Number of strips	Calibration Curve	Sample in Replicate	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips (one plate)	1	42	1 vial	1 vial
8 strips	1	26	700 µl	7.0 ml
6 strips	1	18	500 µl	5.0 ml
4 strips	1	10	350 µl	3.5 ml

Storage after dilution: +2-8°C for four weeks.

7 SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow clotting for 60-120 minutes in room temperature (18-25°C), and separate the serum by centrifugation at 1000-1300 x g for 10 minutes in room temperature.

Note! Hemolyzed serum cannot be used.

Plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction.

Serum and plasma samples can be stored at 2-8°C up to 24 hours.

For longer periods, store samples at –20°C. Avoid repeated freezing and thawing.

7.1 Preparation of samples

Samples should be diluted 1/11 v/v with Sample Buffer. (25 µl sample + 250 µl Sample Buffer)

8 TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a calibration curve for each assay run.

1. Prepare sufficient microplate wells to accommodate Standards and samples in duplicate.
2. Pipette 25 µl each of Sample Buffer, Standards and samples into appropriate wells.
3. Add 100 µl Assay Buffer to each well.
4. Incubate on a plate shaker for 1 hour at room temperature (18-25°C).

5. Wash plate 6 times with automatic plate washer or:
   Aspirate the reaction volume and fill each well completely with 350 µl Wash Buffer.
   Discard liquid completely. Repeat 5 times.
   After final wash, invert and tap the plate firmly against absorbent paper.
6. Add 100 µl Enzyme Conjugate Solution to each well.
7. Incubate on a plate shaker for 1 hour at room temperature (18-25°C).
8. Wash as described above. After final wash, invert and tap the plate firmly against absorbent paper.
9. Add 200 µl Substrate TMB.
10. Incubate for 15 minutes at room temperature (18-25°C).
11. Add 50 µl Stop Solution. Place plate on a shaker for approximately 5 seconds to ensure mixing.
12. Read optical density at 450 nm and calculate results. Read within 30 minutes.

9 INTERNAL QUALITY CONTROL

Internal serum pools with low, intermediate and high MPO concentrations should routinely be assayed as unknowns, and results charted from day to day.

It is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components, OD values for the Sample Buffer, Standards and internal serum pools.

10 CALCULATION OF RESULTS

Note! Sample Buffer is used as a blank and is not used in calibration curve calculation.

10.1 Computerized calculation

The concentration of MPO is obtained by computerized data reduction of the absorbance for Standards 1 - 5, versus the concentration using cubic spline regression.

10.2 Manual calculation

1. Plot the absorbance values obtained for the Standards 1 - 5, against the MPO concentration on a log-log paper and construct a calibration curve.
2. Read the concentration of the unknown samples from the calibration curve.
3. Multiply the concentration of the unknown samples with dilution factor (e.g. x11)

Example of worksheet

Wells	Identity	A_{450}	Mean conc. µg/l*
1 A-B	Sample Buffer	0.066/0.070	
1 C-D	Standard 1 (3 µg/l)**	0.095/0.089	
1E-F	Standard 2 (10 µg/l)**	0.159/0.169	
1 G-H	Standard 3 (30 µg/l)**	0.347/0.368	
2 A-B	Standard 4 (100 µg/l)**	1.048/1.102	
2 C-D	Standard 5 (300 µg/l)**	2.490/2.635	
2 E-F	Unknown 1	0.181/0.184	131
2 G-H	Unknown 2	0.403/0.443	390
3 A-B	Unknown 3	1.211/1.233	1294

* Result multiplied by dilution factor (x11)

** Exact concentration indicated on vial label.

11 LIMITATIONS OF THE PROCEDURE

As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated. Grossly lipemic or icteric samples do not interfere in the assay.

12 EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values.

13 PERFORMANCE CHARACTERISTICS

13.1 Detection limit

The detection limit is ≤ 3 (µg/l).

13.2 Recovery

Recovery upon addition is 84 – 98.4% (mean 89.4%)
Recovery upon dilution is 101.4 ± 5.0 %

13.3 Precision

Each sample was analyzed in 4-replicates on 33 different occasions

		Coefficient of variation		
Sample	Mean value (µg/l)	within assay %	between assay %	total assay %
1	11.58	4.4	9.7	9.9
2	34.93	3.0	8.5	8.6
3	119.21	3.1	5.3	5.5

13.4 Specificity

The following cross-reactions have been found:

TPO	≤ 0.01%
CRP	≤ 0.01%
EPO	3.53%
Lysosym	0.03%
Elastas	0.12%
alfa 1-antitrypsin	≤ 0.01%

14 CALIBRATION

MPO ELISA kit is calibrated against a highly purified, fully validated, commercial MPO preparation. The concentration of myeloperoxidase is expressed in µg/l.

15 WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by DRG® AB may affect the results, in which event DRG® AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. DRG® AB and its authorized distributors, in such event, shall not be liable for damages indirect of consequential.

16 REFERENCES

1. Baldus S. et al. Myeloperoxidase Serum Levels Predict Risk in Patients With Acute Coronary Syndromes. Circulation 2003, 108:1440-1445
2. Brennan M-L. et al. Prognostic value of Myeloperoxidase in Patients with Chest Pain. The New England Journal of Medicine 2003, 349:1595-1604
3. Carr A C. et al. Oxidation of LDL by Myeloperoxidase and Reactive Nitrogen Species. Arterios¬cler Thromb Vasc Biol 2000, 20:1716-1723
4. Nambi V. The Use of Myeloperoxidase As a Risk marker for Atherosclerosis. Current Atherosclе¬rosis Reports 2005, 7:127-131.
5. Hazen S L. et al. Formation of Nitric Oxide-Derived Oxidants by Myeloperoxidase in Monocytes. Circ. Res. 1999, 85:950-958.

6. Zhang R. et al. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA. 2001, 286:2136-2142.

17 SUMMARY OF PROTOCOL SHEET

Add Sample Buffer, Standards, and Samples	25 µl
Add Assay Buffer	100 µl
Incubate	1 hour at 18-25°C on a plate shaker
Wash plate with Wash Buffer	6 times
Add MPO Enzyme Conjugate Solution	100 µl
Incubate	1 hour at 18-25°C on a plate shaker
Wash plate with Wash Buffer	6 times
Add Substrate TMB	200 µl
Incubate	15 minutes
Add Stop Solution	50 µl Shake for 5 seconds to ensure mixing
Measure $_{A450}$	Evaluate results

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SYMBOLS USED WITH DRG® ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke		Sólo para uso en investigación	
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
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